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Trisomy 7 CVS Mosaicism: Pregnancy Outcome, Placental and DNA Analysis in 14 Cases

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Prenatal diagnosis by chorionic villus sampling (CVS) documents placental chromosomal mosaicism in approximately 2% of viable pregnancies at 9–12 weeks of gestation and can involve various chromosomes and placental cell lineages. Confined placental mosaicism (CPM) is the result of postzygotic mitotic errors occurring in either diploid or trisomic zygotes. With trisomic zygote rescue, depending on the parental origin of the chromosome which is lost, uniparental disomy (UPD) or biparental disomy (BPD) may arise [Kalousek et al., Am J Hum Genet 52: 8–16, 1993].

In this paper, we present 14 pregnancies which were diagnosed by CVS as mosaic trisomy 7. All follow-up amniocenteses showed a normal diploid karyotype. Using both classical cytogenetics and interphase analysis, studies of term placentae showed variable levels of trisomy 7. DNA analysis was performed in nine cases to determine whether the diploid fetus had BPD 7 or UPD 7. Fetal UPD 7 was present only in one case; in eight other cases biparental inheritance was demonstrated. DNA analysis to establish the origin of trisomy 7 in the placenta was fully informative in six cases. One trisomy resulted from a meiotic error and was associated with fetal UPD 7, while the rest were somatic in origin.

It is difficult to compare the effect of CPM for trisomy 7 to other trisomies confined to the placenta, as for most chromosomes there are few available cases. It appears that intrauterine fetal growth is not greatly affected by the presence of a trisomy 7 cell line in the placenta. This finding is in contrast to the serious effect of high levels of trisomy 16 within the placenta on fetal intrauterine growth in a series of well-documented cases of CPM 16 [Kalousek et al. 1993].

KEY WORDS: CPM trisomy 7, meiotic and mitotic origin, fetal UPD 7

INTRODUCTION

Mosaicism is typically defined as the presence of two or more karyotypically different cell lines within an individual. The events which initiate mosaicism occur early in embryogenesis and result in either generalized or confined mosaicism. Confined placental mosaicism (CPM) is a discrepancy between the chromosome complement of the embryo/fetus and the placenta, and occurs in 1-2% of pregnancies evaluated by CVS at 9–12 weeks gestational age [Wang et al., 1993].

CPM may be due to the loss of one chromosome in the embryonic progenitors of the conceptus arising from a trisomic zygote or due to somatic duplication of a whole chromosome in placental progenitors originating from a diploid zygote. A complete dichotomy between the placenta and the fetus is indicative of trisomic zygote rescue and fetal UPD is possible [Engel, 1980]. Fetal UPD for some chromosomes is associated with specific clinically determined phenotypes such as Prader-Willi and Angelman syndromes with maternal or paternal UPD of chromosome 15 [Malcolm et al., 1991; Bottani et al., 1994; Nicholls et al., 1989, Robinson et al., 1991; Mascari et al., 1992; Woodage et al., 1994].

Review of the literature identifies nine infants with UPD for chromosome 7. Most of the patients showed maternal UPD and were ascertained due to clinical diagnosis of recessive diseases such as cystic fibrosis [Spence et al., 1988; Voss et al., 1989] or a metabolic disorder [Spotila et al., 1992]. Recently, 4 of 35 patients

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with either the Silver-Russell syndrome or primordial growth retardation were found to have maternal UPD 7. Three of these were completely isodisomic and one showed heterodisomy [Kotzot et al., 1995]. Postnatal growth retardation has been found as a main manifestation in all cases of maternal UPD 7, although severe intrauterine growth restriction (IUGR) was not uniformly present or commented on. One case of UPD for paternal 7p and maternal 7q has been observed in a growth retarded child [Eggerding et al. 1994]. Paternal isodisomy for the entire chromosome 7 was reported in a child with a recessive condition including congenital chloride diarrhea, but normal growth [Hoglund et al., 1994].

We present the analysis of 14 cases of trisomy 7 CPM diagnosed prenatally by CVS. Clinical data, cytogenetic and DNA analyses were compiled to determine the effect of CPM for trisomy 7 on pregnancy outcome. DNA analysis established the parental origin of the disomic chromosomes in nine infants and the origin of the extra chromosome 7 in placental tissues in six pregnancies.

MATERIALS AND METHODS

We studied 14 pregnancies in which trisomy 7 was diagnosed by CVS and follow-up amniocentesis revealed diploidy in the fetus.

At the time of delivery, the birthweight, gestational age, and phenotypic appearance of the infant was recorded. The term placenta was examined for gross and microscopic abnormalities and sampled for cytogenetic and DNA analysis. In all cases classical cytogenetic analysis was performed on cultured amnion, chorionic plate, chorionic villi, and peripheral blood of the newborn. For each sample 15 metaphases were analysed using trypsin-Giemsa banding. In 12 cases the trophoblast of chorionic villi was examined by fluorescence in situ hybridization (FISH). FISH was performed on digested placental tissue [Henderson et al., 1996] using a modified protocol by Pinkel et al. [1988]. Five hundred interphase nuclei from each biopsy were scored.

DNA was extracted from parental and newborn blood samples in nine cases and in six cases from placental material using standard protocols. DNA analyses of all placental and parental samples was performed using polymerase chain reaction (PCR) amplification of microsatellite polymorphisms. Primers were obtained from Research Genetics, Inc. (Information on primers and map location are available from the Genome Data Base.)

The parental origin of the two chromosomes 7 in the infant/fetus was established by DNA typing of markers D7S513, D7S515, D7S396, D7S482, and D7S483 on both parents and the infant. To determine the origin of the extra chromosome in placenta, the following loci were examined: D7S531, D7S664, D7S691, D7S645, D7S1870, D7S800, and D7S495.

In cases of trisomy of meiotic origin, some loci retain parent of origin heterozygosity, but show reduction to homozygosity at others, due to meiotic recombination. In order to distinguish between a meiosis I and meiosis II origin, centromeric markers are necessary. When all markers on the chromosome pair show reduction to homozygosity it is assumed that the extra chromosome has arisen by a postzygotic duplication mechanism as it is unlikely that all loci would show reduction to homozygosity if the nondisjunction was due to a meiotic event [Antonarakis et al., 1993; Robinson et al., 1993].

RESULTS

Fourteen cases with CPM for trisomy of chromosome 7 were identified by classical cytogenetic analysis of cultured CVS. Amniotic fluid cell cultures in all cases demonstrated a normal fetal diploid karyotype as did analysis of newborn blood. The indication for prenatal diagnosis was advanced maternal age in 13 out of 14 cases. The pregnancy outcomes as well as results of cytogenetic and DNA analysis are given in Table I. No

TABLE I. Prenatal and Postnatal Cytogenetic Analysis, Pregnancy Outcome, and DNA Analysis for Origin of Disomy in Newborn and Origin of Trisomy in Placenta in 14 Pregnancies

	% Trisomy 7				DNA analysis	
		Term placenta				
Case	Cultured CVS	Cultured stroma	Trophoblast	Pregnancy outcome[a]	Type of fetal disomy[a]	Origin of trisomy (meiotic or somatic and parent of origin if known)
1	10	0	—	normal	BPD	Not tested
2	75	75	0	normal	BPD	Somatic
3	88	63	—	normal	BPD	Somatic
4	100	63	93	SGA	UPD	Meiotic
5	35	53	0	normal	BPD	Somatic (paternal)
6	7	0	0	normal	BPD	Not tested
7	27	9	10	normal	BPD	Somatic (maternal)
8	100	33	14	normal	BPD	Somatic (maternal)
9	66	18	11	normal	BPD	Not tested
10	85	48	7	SGA	Not tested	Not tested
11	100	73	16	normal	Not tested	Not tested
12	31	0	0	normal	Not tested	Not tested
13	18	9	0	normal	Not tested	Not tested
14	17	13	0	normal	Not tested	Not tested

[a] SGA, small for gestational age; UPD, uniparental disomy; BPD, biparental disomy.

congenital anomalies were present in any newborn infant. Gross and microscopic examinations of all placentae were unremarkable.

The mosaicism detected in cultured stroma at CVS (9–12 gestational weeks) ranged from 7 to 100%, while trophoblast was not analysed. At 36–40 gestational weeks, 10 placentae showed trisomy 7 by classical cytogenetic analysis of chorionic stroma (9–75%). FISH analysis of trophoblast in 12 placentae showed trisomy 7 mosaicism in 6 of them (7–93%). Six other placentas showed only diploid cell populations in trophoblast and the trophoblast of two placentas could not be studied by FISH. Two cases of fetal IUGR were associated with placental trisomy 7 expressed in both cell lineages (extraembryonic mesenchyme and trophoblast), while all those with diploid trophoblast were associated with normal birthweight.

DNA analysis of parents and newborn infants identified biparental inheritance in eight infants of normal birthweight and maternal heterodisomy in one infant (case 4) presenting with severe IUGR.

Somatic origin of CPM 7 was identified in five placentas. In these placentas three alleles were not observed as would be expected for at least some loci if the trisomy originated as a meiotic error. The absence of a third allele cannot be explained by low level of the trisomic cell line as cytogenetic analysis of the specific tissue samples used for DNA analysis showed 36–84% trisomic cells. It has been estimated that a faint third allele, if present, should be observable with as low as 5–10% mosaicism [Pangalos et al., 1994; Robinson et al., 1995]. The parent of origin could be clearly identified in three placentae by assessing relative densities of the bands. Cases 7 and 8 indicated somatic duplication of the maternal chromosome 7 and case 5 was paternal in origin. Case 4 showed meiotic origin of CPM and coincided with fetal maternal UPD 7 and high levels of trisomy 7 in both placental cell lineages. This infant with maternal UPD 7 has been reported separately [Langlois et al., 1996].

DISCUSSION

In pregnancies with prenatally diagnosed CPM, pregnancy outcomes are variable. The infants may be normal, show IUGR, or die perinatally. The cell lineage(s) involved, the extent of involvement of the placenta and the specific chromosome present as trisomy appear to correlate with pregnancy outcome [Gosden et al., 1995]. For most chromosomes the effect of fetal UPD on pregnancy outcome and postnatal growth and development is not clearly understood.

Trisomy 7 is one of the most common aneuploidies detected by CVS, but has not been described in nonmosaic form in liveborn infants. It has been suggested that maternal non-disjunction is the most likely cause of trisomic zygotes as deduced from parental origin of the extra chromosome in abortion and liveborn trisomy studies [Hassold et al., 1991; Fisher et al., 1995; Antonarakis et al., 1993; Zaragoza et al., 1994]. The survival of a trisomic conceptus can only occur by a mechanism known as trisomic zygote rescue, where a reduction from aneuploid to diploid occurs in the true embryonic progenitor resulting in a diploid fetus supported by a trisomic placenta. Trisomic zygote rescue has been previously identified for chromosomal trisomies 2, 14, 15 and 16 [Bernard et al., 1995; Morichon-Delvallez et al., 1993; 1994; Dworniczak et al., 1992; Bennett et al., 1992; Malcolm et al., 1991; Cassidy et al., 1992; Purvis-Smith et al., 1992; Kalousek et al., 1993; Miny et al., 1994]. The loss of the extra chromosome is thought to be a random process, with a theoretical one in three chance of retaining both chromosomes from one parent, resulting in UPD [Engel, 1980].

In trisomic zygote rescue, high levels of both stromal and trophoblastic trisomy are often observed [Kalousek et al., 1993] and, in this situation, the association is more likely with uniparental disomy in the fetus. In most cases of somatic duplication, the extent of placental mosaicism is more limited and fetal UPD unlikely.

It is generally assumed that approximately one third of CPM cases are at risk of UPD in the normal fetal cell line [Engel and Delozier-Blanchet, 1991]. However, that risk assessment is based on the assumption that all cases of CPM arise from trisomic zygotes, rather than from post-zygotic mitotic duplication of a chromosome. Therefore, the risk of UPD associated with CPM will depend on the frequency with which CPM is due to a meiotic or mitotic origin of trisomy. The relative frequency of involvement of each chromosome in spontaneously aborted trisomic conceptions would likely be indicative of expected frequency of UPD for each chromosome.

Among chromosomally abnormal spontaneous abortions, trisomy 7 is rarely seen in comparison to trisomy 16; however, at CVS, trisomies 7 and 16 CPM occur at similar frequencies. This observation in conjunction with the data presented suggests that most of CPM 7 cases are due to somatic duplication, not trisomic zygote rescue, and the risk of fetal UPD is low. Whereas trisomic zygote rescue is commonly seen in CPM 16 and the risk of fetal UPD is closer to the expected ⅓ [Kalousek et al., 1993].

The present study reports 14 cases with CPM for trisomy 7 and a diploid fetus. There is a wide range of mosaicism observed in the CVS and term placentae. In five out of six cases studied for the origin of placental mosaicism, DNA evidence suggests that the trisomic cell line arose by a somatic duplication event within the placental lineage. This study substantiates the hypothesis that CPM 7 is most frequently due to somatic duplication; therefore, the risk of fetal UPD 7 is low. The single case of fetal UPD 7 identified in this study showed fetal IUGR as reported for UPD 7 in the literature and the origin of CPM was determined to be meiotic.

Maternal UPD 16 in association with CPM for trisomy 16 is often associated with IUGR [Kalousek et al., 1993; Vaughan et al., 1994]. However, CPM for trisomy 16 without UPD was also found to be associated with IUGR, suggesting that the cause of fetal IUGR may be more related to malfunction of trisomy 16 placenta than maternal UPD 16 in the fetus. This study indicates that CPM for trisomy 7 with fetal BPD 7 has no effect on fetal intrauterine growth.

In conclusion, CVS alone is a poor predictor of pregnancy outcome for trisomy 7. When CVS is combined with DNA analysis identifying the origin of CPM, a more reliable prediction of pregnancy outcome can be made. When CPM trisomy 7 is due to a meiotic error, DNA analysis in addition to cytogenetic analysis of amniocytes to determine parental origin of fetal chromosomes 7 should be helpful in predicting the likelihood of fetal IUGR. Long-term follow-up studies of the children from pregnancies with CPM for trisomy 7 to assess postnatal growth and developmental progress are necessary to improve prenatal counselling when mosaic trisomy 7 is diagnosed on CVS.

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